CYTOKINES / CHEMOKINES AT MUCOSAL SURFACES 1
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OR.53. Study on APRIL and AID Distribution in Normal Human Gut

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APRIL is a B cell survival factor linked to T cell independent class switch (CSR) to IgA2 in colonic lamina propria (LP) via AID activation. Here we address the expression of APRIL in human GALT v.s. LP and evaluate its functional significance by studying, in parallel, AID distribution at these sites. Materials and Methods: The distribution of APRIL, AID, CD20, CD68 and neutrophil elastase was assessed in paraffin sections of normal human gut using immunohistochemistry. The relative quantities of RNA encoding APRIL and AID were also analysed in colon and ileum using RT-PCR. Results: In the GALT, APRIL was detected in the dome region, in the T cell zone and in the inner part of the germinal centres (GC). The majority of APRIL positive cells in the GALT were identified as CD68+ macrophages and dendritic cells. In the GC CD68 + tingible body macrophages express APRIL. We frequently observed APRIL expressing cells adjacent to CD20+ B cells, but no double positive APRIL/CD20 cells. AID expression was confined to the nuclei of GC cells and the cytoplasm of large interfollicular B cells. Distant from GALT, APRIL expression was also observed in LP and epithelium, in particular at the bases of the crypts. APRIL expression in LP was predominantly in neutrophils and macrophages. The commonly used antibody to AID showed a tendency to bind non-specifically to seroid LP, being not adequate for AID detection at these sites . qPCR analysis of gene expression largely confirmed the immunohistochemical findings, and in addition identified expression APRIL receptors TACI and BCMA in GALT and gut LP. Conclusions: APRIL and its receptors are expressed abundantly in GALT, in microanatomical compartments positive for AID, demonstrating that GALT houses factors known to support CSR through T dependent and independent mechanisms.

OR.54. A Role for TSLP in the Regulation of Intestinal Immunity and Inflammation

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Intestinal epithelial cells (IECs) have emerged as critical regulators of innate and adaptive immune responses within the intestine. While IECs are known to produce TSLP, the *in vivo* functions of TSLP in regulating intestinal immunity and inflammation remain poorly defined. We have previously demonstrated a critical role for TSLP in immunity to the intestinal pathogen *Trichuris*. Further, TSLP is important in the limitation of infection-induced intestinal inflammation in this model. Here we show that disruption of the TSLP-TSLPR pathway either through neutralization of TSLP or genetic deletion of the TSLPR alters the basal regulation of IL-12/23p40 and IL-17A production in the intestine. Consistent with this, TSLPR-/- mice displayed elevated production of IL-12/23p40 and IFN-γ, and developed heightened intestinal inflammation in a murine model of colitis. Collectively these data suggest that in addition to a role in the promotion of Th2 cytokine responses in the intestine, TSLP has an immunoregulatory role limiting proinflammatory cytokine production and inflammation. This work was funded by the NIH (AI61570, AI74878, F32-AI72943, T32-CA09140-30), Burroughs Wellcome Fund, and the CCFA.

OR.55. Absence of RANKL in Mice Causes Perturbed Development of Cryptopatches and a Complete Block in Formation of Isolated Lymphoid Follicles

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RANKL plays a central role in the development of organized lymphoid structures. RANKL null mice lack lymph nodes and their Peyer's patches (PP) are nearly completely devoid of M cells due to absence of RANKL expression by subepithelial dome stromal cells. Cryptopatches (CP) and isolated lymphoid follicles (ILF), referred to in aggregate as solitary intestinal lymphoid tissue (SILT), are organized lymphoid tissues that develop postnatally in mouse small intestine (SI) and also contain stromal cells that normally express RANKL. In this study we characterized the development of CP and ILF in RANKL null mice. A 4-fold reduction in the overall density of CP was observed in RANKL null mice, with the number of proximal SI CP reduced the most. Also, stromal cells in CP from RANKL null mice were phenotypically abnormal, expressing unusual combinations of stromal antigens. RANKL null mice had a more pronounced defect in ILF development. No ILF were detected, even in RANKL null mice treated in utero with LTβR-Ig to block PP development and accentuate subsequent postnatal ILF development. These results reveal that RANKL is critical for normal CP development and plays an essential role in the maturational pathway through which some CP transition into ILF.

OR.56. Activation of the IL-17 Promoter Directly in T Cells by IRF-4 Contributes to T Cell-dependent Experimental Colitis

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In our project, we study the functions of interferon regulatory factor 4 in intestinal inflammation. We found out that RAG2-/- mice reconstituted with IRF4-/- CD45Rbhigh T cells showed less colonic inflammation, associated with less IL-6 and IL-17 production than RAG2-/- mice reconstituted with IRF4+/+ CD45Rbhigh T cells. After analysing the sequences of mouse IL-6 and IL-17 promoters, we found out possible IRF-4 binding sites within these two promoters. Chromatin

immunoprecipitation suggested a direct IRF-4 binding position on IL-17 promoter. Electrophoretic mobility shift assay further verified this binding site. *In vitro*, recovery of IRF-4 expression increased IL-17 production. Additionally, using colon biopsies of patients with IBD we found out a strong correlation between IRF4 and IL-17 mRNA levels in colon. In contrast, DSS-treated IRF-4-/- mice showed similar colon inflammation as the wild type mice. This maybe because of the involvement of epithelial cells and antigen presenting cells, but not T cells in this model. The findings above suggest that IRF-4 plays a crucial role in chronic intestinal inflammation, and works at least to a certain extent through its directly regulation of Th17 differentiation.